extent with 1.0 μM Ca^{2+} than control RyR2 (P_o was 5X greater in diabetic RyR2). Two weeks of insulin treatment blunted the enhanced Ca^{2+} responsiveness. When added to the *cis* chamber the potent reactive carbonyl species (RCS), 80 μM methylglyoxal increased the open probability (P_o) of RyR2 3-fold (0.05 to 0.16) within 10 min and this increase was independent of holding potential. Increasing [MGO] further to 160 μM, reduced the conductance of RyR2 by 25% without changing P_o. Incubating RyR2 with MGO (5-500 μM with 200 μM free Ca^{2+} in buffer) dose-dependently reduced its ability to binding [3H]ryanodine. Singly mutating R1611, R2190 and K2888 to W or Y, to mimic adducts previously found on them during diabetes, resulted in gain-of-function of RyR2 (P_o increased >2-fold at 3.3 μM Ca^{2+}). Mutating c-terminal R4462, and R4683 to W or Y resulted in loss-of function of RyR2. We conclude that modification of RyR2 by RCS during diabetes is responsible in part for its dysregulation. (This work was funded by NIH and Nebraska Redox Biology Center)

1573-Pos

Gating of the Purified Human Cardiac Ryanodine Receptor (hRyR2) in the Absence of Regulatory Accessory Proteins

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The cardiac ryanodine receptor (RyR2) mediates Ca^{2+} efflux from intracellular stores to effect myocyte contraction during excitation-contraction coupling. Mutations in this channel perturb Ca^{2+} release function, leading to triggered arrhythmias that may cause sudden cardiac death (SCD). The exact molecular mechanisms by which SCD-linked RyR2 dysfunction occurs constitutes a burgeoning area of cardiac research. Most studies so far have concentrated on the secondary effects of mutation on channel function by virtue of affecting channel modification by phosphorylation and accessory protein binding, with no great emphasis on elucidating the gating mechanisms of the channel itself. Our aim is to elucidate the mechanistic basis of wild-type (WT) hRyR2 activation by its primary activating ligand, Ca^{2+}, under precisely controlled conditions in the absence of any accessory proteins with a view to determining the effect of mutation on hRyR2 gating in the same way. hRyR2 channels, recombinantly expressed in HEK293 cells, were purified and studied at the single channel level in symmetrical 210mM KCl under reducing conditions. *Trans* (luminal) Ca^{2+} was buffered at 50nM using EGTA, while *cis* (cytosolic) Ca^{2+} buffering was stringently controlled using EGTA, HEDTA and NTA to achieve free Ca^{2+} concentrations in the range of 0-500 μM. Preliminary data obtained from sigmoidal dose-response curves of P_o vs pCa for 10 WT hRyR2 channels yields an EC_{50} of 3.25 ± 1.04 μM, resulting in a maximum P_o greater than 0.8 (in 5 out of 10 channels). This increase in P_o resulted from an increase in the frequency of channel openings, until P_o of 0.8 - above which any increases in P_o resulted from an increase in open times. Likely gating mechanisms will be discussed with a view to mutant channel analysis.
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1574-Pos

S-Adenosyl-L-Methionine Activation of Cardiac Ryanodinr Receptors is Associated with an Increased Frequency of Subconductance Sates

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The biological methyl group donor, S-adenosyl-L-methionine (SAM) activates the cardiac ryanodine receptor (RyR2). Previously we investigated the mechanism underlying SAM regulation of RyR2 with [^{3}H]ryanodine binding to cardiac SR vesicles. SAM enhances Ca^{2+} -activation of RyR2 and increases the apparent affinity of ryanodine for the channel. Notably, methyltransferase inhibitors have no effect on SAM-activation, and SAM-mediated methylation of RyR2 is not detected. Furthermore, the concentration dependence for SAM and ATP-induced increase in [^{3}H]ryanodine binding overlap. Presently, we investigated the affect of SAM on native RyR2 channels incorporated into planar lipid bilayers. Channel were grouped according to initial P_o values under control conditions (10 μM cytosolic Ca^{2+}), those with $P_o < 0.2$ (n=7), and $P_o > 0.2$ (n=5). For channels with an initial $P_o < 0.2$, SAM caused a rapid (within seconds) increase in P_o ($p < 0.05$). The SAM-induced increase in P_o was due primarily to an increase in mean open time ($p<0.05$; n=3). Interestingly, SAM activation was associated with an increased frequency of subconducatance states. In contrast, the increase in channel P_o caused by 2mM ATP was not associated with the appearance of subconductance states. Thus, the effect on subconductance states appears specific to SAM. This work highlights the complexity underlying SAM regulation of RyR2. The data suggest ligand binding is among the multiple mechanisms responsible for SAM-activation of RyR2.

1575-Pos

Molecular Interplay between the Heart Lim Protein (HLP) and RyR2 in Murine Heart

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HLP is a heart-specific LIM-only protein having two LIM domains each consisting of two zinc fingers. Through the bacterial 2 hybrid screening and a following LC-MS/MS study, we have found that HLP interacts with the cytosolic divergent region of mouse heart RyR2. The direct interaction between RyR2 and HLP was confirmed by GST pull-down and co-immunoprecipitation assays. HLP was partially co-localized with RyR2 in HL-1 cells and rat adult cardiomyocytes. siRNA or Adenovirus-mediated knock-down of HLP in HL-1 cells and neonatal cardiomyocytes led to more than 70% decrease in the expression of HLP, without a concomitant change of other Ca^{2+} handling proteins (e.g. SERCA, RyR2, calsequestrin and DHPR). Ca^{2+} transient measurement of fura2-loaded cardiomyocytes by 1Hz field stimulation demonstrated that silencing of HLP decreased the peak amplitude of Ca^{2+} transient (~ 15%) in HL-1 cells and in neonatal cardiomyocytes. Currently, various deletion-mutants of LIM protein are being used to characterize the RyR2 binding sites in HLP. (This work was supported by the Korean Ministry of Science and Technology grant, Systems Biology Research Grant, M1050301001-6N0301-0110, and the 2009 GIST Systems Biology Infrastructure Establishment Grant).

1576-Pos

Mitsugumin-29 Regulates RyR1 Activity In Mouse Skeletal Myotubes

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Canonical-type transient receptor potential cation channel type 3 (TRPC3) in plasma membrane allows the entry of Ca^{2+} ions into various cells. In skeletal myotubes, functional interaction between TRPC3 and RyR1 (ryanodine receptor1, a Ca^{2+} channel in sarcoplasmic reticulum (SR) membrane) regulates the gain of skeletal excitation-contraction coupling (*J. Biol. Chem*., 2006). Mitsugumin-29 (MG29) is a four membrane-spanning protein and is found in both plasma and SR membrane. MG29 has been known as a TRPC3-interacting protein in skeletal myotubes (*Biochem. J.*, 2008).
To identify critical region(s) of MG29 that participate in binding to TRPC3 or the role of MG29 in skeletal muscle, N-terminus, three intervenient loops among four transmembrane regions, and C-terminus of MG29 were expressed in E. coil as N-terminal GST-fused forms, and subjected to co-immunoprecipitation assay with intact TRPC3 from rabbit skeletal muscle. Cytoplasmic N-terminus and a loop between first and second transmembrane domains of MG29 effectively bound to TRPC3. Two deletion mutants of MG29 (missing the TRPC3-binding sites: deleting the N-terminus only or longer N-terminus covering the loop region) was expressed in mouse skeletal myotubes, and the myotubes was subjected to the measurement of Ca^{2+} transients with Fura-2 or Fluo-4. The later mutant showed significantly decreased responsiveness of RyR1 to caffeine, suggesting that MG29 may be a mediator between the functional interaction between TRPC3 and RyR1.

1577-Pos

Phosphorylation of Excitation-Contraction Coupling Components in a Guinea-Pig Model of Heart Failure

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Phosphorylation status appears to be a key determinant of excitation-contraction coupling ion channel and pump function. Dysfunction of the ryanodine receptor (RyR) secondary to catecholaminergic drive and phosphorylation has been proposed as a factor in contractile dysfunction and arrhythmia patho-physiology in the failing heart. The phosphorylation states of RyR, along with those of phospholamban and troponin I have been investigated by immunoblotting, and quantitated by comparing levels in failing hearts with basal levels, minimum levels after beta-blocker treatment and maximal levels achieved by ex vivo treatment with isoprenaline. We found that RyR residue Ser2809 was phosphorylated to 124 ± 11 % ($n = 5$, $P > 0.05$) of control (sham-operated, basal) in heart failure under basal conditions and 143 ± 12 % ($n = 6$, $P < 0.05$) with isoprenaline treatment, and residue Ser2030 was 94 ± 10 % ($n = 8$, $P > 0.05$) for heart failure and 199 ± 9 % (n=6, $P < 0.05$) for isoprenaline treatment. Phosphorylation levels at Ser16 of phospholamban were higher: 159 ± 17 % (heart failure, $n = 7$, $P < 0.05$) and 366 ± 95 % (isoprenaline treatment, $n = 5$, $P < 0.05$). At Ser23/24 of troponin I there is no significant